# OBSERVATIONS ON GREEN PRODUCING COCCI OF INFLUENZA

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During the recent (1918-1919) influenza epidemic the majority of observers isolated diplococci from the sputum and from material obtained at necropsy. The organisms were classed generally as pneumococci of type 4 on account of their being soluble in bile and not agglutinated by pneumococcus serums of types 1, 2 and 3. Pneumococci of types 2, 3, and more rarely of type 1, were also isolated. Other workers, notably Mathers,[1] MacDonald,[2] Rosenow,[3] Howell and Anderson,[4] Jordan,[5] isolated bile-insoluble diplococci or streptococci from the nasopharynx, sputum, blood and necropsy material, which in 24 hours produced large, flat, moist, green colonies on blood-agar plates. MacDonald describes his colonies as slightly hemolytic. Rosenow [6] says his strains acquired hemolytic power. Howell and Anderson describe their cocci as nonhemolytic during the 10 days in which they were observed. Bernhardt and Meyer [7] isolated a bile-insoluble, gram-positive diplococcus, "Diplococcus epidemicus," which on human agar produced very minute, slightly hemolytic colonies with a black-greenish tinge. In 1917 Stephan [8] described as the agent of a clinically typical endemic of influenza in Leipzig a "diplococcus mucosus," which he isolated from the sputum and the blood, and which was agglutinated specifically by an immune serum as well as by patient and convalescent serum. It produced a capsule in the body and mucus in some mediums, was variable in its retention of Gram's stain, nonpathogenic to animals, insoluble in bile, but not identical with Str. mucosus of Schottmüller or pneumococcus mucosus. As the original article cannot be obtained now it is impossible to say whether this diplococcus and the coccus

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[1] See Tunnicliff: Jour. Am. Med. Assn., 1918, 71, p. 1723.
[2] Brit. Med. Jour., 1918, 2, p. 481.
[3] Jour. Am. Med. Assn., 1919, 72, p. 31.
[4] Jour. Infect. Dis., 1919, 25, p. 1.
[5] Ibid., p. 28.
[6] Jour. Am. Med. Assn., 1919, 72, p. 1604.
[7] Med. Klin., 1918, 14, p. 683; abst., Jour. Am. Med. Assn., 1918, 71, p. 1573.
[8] München. med. Wchnschr., 1918, 65, p. 257; abst., Jour. Am. Med. Assn., 1918, 71, p. 1573.

described by Mathers are identical. Howard [9] isolated green streptococci from the majority of his cases of influenza, but found the colonies smaller and less moist than typical pneumococcus colonies, and not different from colonies isolated from control cases. Cocci described as Str. viridans and nonhemolytic streptococci were isolated by Keegan,[10] Nuzum,[11] Blanton and Irons,[12] Deven,[13] Meyer,[14] Dick and Murray [15] and others, but the colonies are not described in such a way that they may be compared with those of other observers.

In the observations under consideration solubility in bile is the main criterion whether a coccus belongs to the pneumococcus or streptococcus groups, but unfortunately, as a rule, no description is given of the methods used in determining bile solubility. The ability of bile to dissolve influenzal cocci is recorded differently by different workers. Keegan [10] found it difficult to differentiate pneumococci of type 4 from Str. viridans, the bile test of broth cultures not being found reliable. Nuzum [11] found that pneumococci of type 4 and allied green producing organisms varied greatly as regards bile solubility. Dunn [16] also mentions that the bile solubility of his cultures of diplococci varied, 40% being soluble. Some of Howard's [9] strains were partially bile soluble.

On account of the difficulty in differentiating these organisms by the bile test, I have made immunity experiments to determine particularly whether various green-producing cocci, isolated from influenza and its complications, soluble and insoluble in bile, are in any way related.

I have shown [1] that specific opsonin for the peculiar green-producing streptococci from influenza developed during the course of the disease. A specific opsonic decrease was observed in the pneumonia following influenza, which persisted unless the patient recovered, when the opsonins rose to normal or above. No agglutinins could be demonstrated in the case of 3 strains tested, with the serum of 5 influenza and 2 pneumonia patients examined during the attack and convalescence. On the other hand, Rosenow found that the serum of patients convalescent from influenza agglutinated specifically some of the more sensitive of his green-producing streptococcus strains, but that some

[9] Bull. Johns Hopkins Hosp., 1919, 33, p. 13.
[10] Jour. Am. Med. Assn., 1918, 71, p. 1051.
[11] Ibid., p. 1562.
[12] Ibid., p. 1988.
[13] Ibid., 1919, 72, p. 265.
[14] Cal. State Jour. Med., 1919, 17, p. 216.
[15] Jour. Infect. Dis., 1919, 25, p. 6.
[16] Jour. Am. Med. Assn., 1918, 71, p. 2128.

strains were not agglutinated at all. A monovalent horse serum prepared by him with one of these strains specifically agglutinated 65, that is 66%, of 98 strains studied, most of the negative results being obtained with cultures made during convalescence. Other streptococci from a wide range of sources were rarely agglutinated by this serum. As stated, many of his strains acquired hemolytic power on cultivation. The close relationship of the green-producing strains to hemolytic streptococci was indicated, moreover, he says, by the fact that hemolytic streptococcus serum commonly manifested decided agglutinating power for the green producers. He concludes that his experiments show that green-producing strains of streptococci or diplostreptococci from influenza are immunologically identical or closely related. The results of his absorption tests were in harmony with this conclusion.

Howell and Anderson [4] obtained a large number of fixation reactions with influenza serums and strains of green-producing streptococci from influenza at Camp Meade and in Chicago. The serum of the convalescent influenza patients came from a wide range of localities.

Here it may be noted that various observers have tried to differentiate green-producing streptococci into groups by various immunity reactions, the opsonic probably being the most successful. Kinsella and Swift [17] believed they could classify nonhemolytic streptococci by complement fixation tests, but Howell [18] concluded that the organisms could not be classified by that method. Agglutination tests were found useful by Rosenow and Gray [19] for differentiating the poliomyelitis streptococcus from streptococci and pneumococci from other sources. Mathers and Howell [20] obtained better results in differentiating the poliomyelitis coccus with immunized rabbit serum when opsonins were studied rather than agglutinins, on account of spontaneous agglutination. Nuzum and Willy [21] found that by means of opsonic determinations of immune horse serum, they were able to separate strains of poliomyelitis cocci from other strains of cocci presenting more or less confusing cultural and morphologic similarities. Later Davis [22] found well-marked specific increase in opsonins for the poliomyelitis streptococcus in the serum of monkeys with experimental poliomyelitis. Opsonic determination of specific immune rabbit serum

[17] Jour. Exper. Med., 1917, *25*, p. 877.
[18] Jour. Infect. Dis., 1918, *22*, p. 230.
[19] Ibid., 1918, *22*, p. 345.
[20] Ibid., 1917, *21*, p. 292.
[21] Ibid., 1918, *22*, p. 258.
[22] Ibid., 1919, *24*, p. 176.

was found by Tunnicliff and Brown [23] to be an easy and reliable method to differentiate the diplococci found in measles and rubella from each other and from other nonhemolytic diplococci and streptococci. These cocci when first isolated were not suitable for agglutination tests because of spontaneous clumping.

The thirty-four strains of green producing cocci from influenza used in these experiments were isolated by the late Captain George Mathers at Camp Meade, by Major E. F. Hirsch at Camp Grant, by Dr. Nuzum in Chicago, and by myself at Camp Meade and in Chicago. All of these strains were isolated during the early months of the epidemic (September, October and November, 1918).

Bile solubility was determined by adding one-fifth the volume of sterile ox-bile to a broth culture; the mixtures were incubated for two hours as some strains were found to dissolve slowly in bile, and compared macroscopically with a broth culture without bile. Suspensions of the cocci in salt solution gave results similar to those in broth cultures. Of the 34 strains, 23 were insoluble in bile and consequently would be classed as streptococci, but seven of them were agglutinated by pneumococcus serum of types 2 and 3 (Rockefeller). The 11 bile-soluble strains belonged to pneumococcus groups of types 2, 3 and 4, according to their agglutination reactions.

All of the 34 strains produced green colonies on goat blood agar, and after 48 hours' incubation and 24 hours' refrigeration showed partial hemolysis of the corpuscles immediately surrounding the colony. The majority of the strains produced large, moist colonies, similar to the colonies described by Mathers.[1] The Camp Grant cultures, as a rule, gave rise to smaller and drier colonies than the others. Some of the strains which on isolation produced large, moist colonies, after six months cultivation grew in much smaller and less moist colonies, but without change in their hemolyzing power.

The eight strains which were agglutinated by pneumococcus serum of type 3 were moister than the other strains, but not mucoid. These cocci were larger and rounder and possessed a wider capsule than the others.

The strains isolated by me in Chicago were obtained from the edematous brain in ten cases of influenzal bronchopneumonia in adults. The brains were removed by Dr. E. R. LeCount immediately after death, the surface seared, the brain substance broken up and removed with a large sterile pipet and the material added to broth, cultures being made from this mixture. At first both aerobic and anaerobic cultures were made on human blood agar, but as the same organisms were found in both, the anaerobic cultures were discontinued. The blood-agar plates were made by adding 5% blood to 12 c c beef extract agar (Fairchild Culture Peptone), which was 0.8% acid to phenolphthalein. After the original culture was made goat blood was used instead of human. The cultures from one brain were sterile. Gram-positive diplococci in medium long chains were grown from the other

[23] Ibid., 1918, 23, p. 572; 1919, 24, p. 181.

9, in pure culture except for staphylococci in 2 broth cultures which also contained a rapidly growing bacillus. In no instance was the Pfeiffer bacillus cultivated. Twice the cocci grew in the original broth culture, but not on the aerobic blood-agar plate. Two strains would not grow in subculture, possibly due to their not being transferred at the end of 24 hours' incubation. From 5 to 10 colonies grew on the original plates. The 2 strains of diplococci isolated in broth and then grown on blood agar, produced green colonies, which were drier and smaller than those isolated from the spinal fluid of the same patient in one case and from the brains and fluids of the other 5, grown originally on blood agar. After 24 hours' incubation these 6 strains produced on blood agar, large, round, moist, rather flat, green colonies, 2-4 mm. in diameter, with regular edges, which had a tendency to become confluent. The colonies often showed umbilication after longer incubation. The green discoloration was only under the colony. After 24 hours' incubation and 24 hours' refrigeration, a narrow, very slight hemolytic area was seen around the edge of the colony and a similar zone just inside the colony, giving the appearance of three rings. The hemolysis corresponds to the alpha type of Smith and Brown.[24] Plates were made according to Brown's[25] method: 5% blood agar (12 c c) was inoculated with a 24-hour broth culture and poured into a Petri dish, 9 cm. in diameter. After 48 hours' incubation and 24 hours' refrigeration the colonies appeared biconvex, the deep colonies being about 0.5 mm. in diameter, with a somewhat greenish discoloration and partial hemolysis of the blood corpuscles immediately surrounding the colony for about 0.5 mm., and surrounded by a second, clearer, not discolored, partly hemolyzed zone of about the same diameter. When incubated 48 hours and refrigerated, the characteristic appearance seen on the surface of blood-agar plates after 24 hours' incubation was lost.

The same green-producing cocci were isolated in pure culture from the blood, pleural, pericardial and spinal fluids in two cases. In a third case, that of a pregnant woman, the placental and fetal blood were sterile.

Of the 7 strains studied, only 1 was soluble in bile. This bile soluble culture was not agglutinated by pneumococcus serums of types 1, 2 and 3.

[24] Jour. Med. Research, 1915, 13, p. 455.
[25] Monograph No. 9, Rockefeller Inst. for Med. Research, 1919.

All 7 strains fermented glucose, lactose, saccharose and raffinose. Six fermented salicin, 2 inulin and 2 mannite. Hiss' sheep serum water was used and the cultures were incubated one week. The cocci produced little sediment in 1% glucose broth, except one culture.

Morphologically, the organisms were generally pointed diplococci with a capsule. Some were distinctly round, but the shape varied with cultivation. Some cultures produced longer chains than others.

The virulence of these organisms was not tested when first isolated. Two of the 4 strains tested were virulent at the end of 3 months, $\frac{1}{200}$ of a 24-hour broth culture killing a mouse within 24 hours. These two organisms had been transplanted about once a week during the three months.

According to our present method of classification, the bile insoluble strains would be called streptococci. The bile soluble strain, which would be classed as pneumococci of type 4, was otherwise like the insoluble strains. Immunologically, they were found to react alike. Rabbits immunized with the bile insoluble streptococci isolated from sputum and pneumonic lungs in influenza produced opsonins and agglutinins for all of these brain strains. These experiments are discussed later.

Apparently the brain cocci are like the cocci isolated by Mathers and others from influenza and its complications. The colonies closely resemble those of the Str. viridans isolated by Zingher[26] from empyema and from the lung, but the cocci differ in possessing a capsule and in not as a rule growing in long convoluted chains in broth, leaving the fluid clear.

## IMMUNITY EXPERIMENTS

As opsonin is the antibody most easily demonstrated for streptococci, and agglutinin the antibody generally used to differentiate pneumococci, observations were made on both these antibodies.

Rabbits were immunized in various ways with different strains of the peculiar green producing cocci from influenza and its complications. At first they were inoculated intravenously about every 4 days with increasing doses of living cocci grown on goat blood-agar slants, the largest dose given being the growth on 4 slants. The rabbits bore the inoculations badly, several dying before immune bodies were demonstrable. Some rabbits were inoculated with killed cocci, at first subcutaneously, later intravenously, and then with living organisms. One rabbit received only killed cocci. For the tests the cocci were transplanted from goat blood-agar to 1% glucose broth and incubated at 24, or 48 hours, if necessary, for growth.

[26] Jour. Am. Med. Assn., 1918, 71, p. 451; 1919, 72, p. 1020.

The opsonic tests were made as follows: At first the serums were used unheated and undiluted, or diluted 1:4 with normal salt solution, and then equal parts of washed human leukocytes and bacterial suspensions were added. It was sometimes necessary to dilute the broth culture suspension if the growth was profuse. Later the point of opsonic extinction of the serums was determined; the results of the two methods were similar. The specimens were incubated at 37 C. for 15 minutes, smears stained with carbol thionin, 50 polymorphonuclear leukocytes counted, and the number taking part in phagocytosis noted.

Later the normal and immune rabbit serum was first heated for one-half hour at 56 C. to destroy the thermolabile element, and the serums then diluted to the point of opsonic extinction, the mixtures of serum, leukocytes and cocci then being incubated for 25 minutes. The results were on the whole the same as with unheated serum. It is important to note that heated and unheated normal serum as a rule contained practically no opsonins at 1:3 for these cocci.

Agglutination was studied by mixing equal parts of serum and broth culture in capillary pipets, incubating at 37 C. for 2 hours and refrigerating 20 hours when the results were read macroscopically (Lister).

The antibodies were produced slowly. Three subcutaneous injections of large numbers of killed organisms (the growth on 4-8 blood-agar slants) produced no agglutination in most instances and not much opsonin. Some rabbits so injected died after receiving a small number of living cocci. Agglutinins as a rule were produced several weeks later than the opsonins, never in high dilutions and generally in much lower dilutions than the opsonins. A few strains agglutinated spontaneously and therefore could not be used in the agglutination tests though suitable for the opsonin determinations. The strains differed in their power to produce agglutinins, and in some rabbits these antibodies appeared earlier than in others. The apparent ready response on the part of the rabbit with opsonins and slow response with agglutinins is in accord with my results with influenza serum which gave marked increase in opsonins for the cocci while no agglutination could be demonstrated.

The serum of the immune rabbits 1 and 2 (table 1) which was tested undiluted or diluted 1:4 caused marked phagocytosis of the influenza cocci — an average of six times more phagocytosis than normal rabbit serum. The serums of the other rabbits which were heated and diluted opsonified the homologous coccus in dilutions as high as 1:11,200; the weakest serum gave phagocytosis with the homologous strain at 1:60. The average dilution at which phagocytosis ceased for heterologous strains was between 1:60 and 1:120. Normal serum, as a rule, showed no opsonins for the strains at a dilution of 1:3, the dilution reached by the addition to the serum of leukocytes and bacterial suspension.

TABLE 1

OPSONINS AND AGGLUTININS IN THE SERUM OF RABBITS IMMUNIZED WITH THE PECULIAR GREEN PRODUCING STREPTOCOCCI FROM INFLUENZA

| Serums | Cocci | |
|---|---|---|
| | Percentage of Peculiar Green Producing Influenza Cocci for which the Serum Showed Definite Increase in Opsonin and Agglutinin | Control Tests with Green Producing Streptococci from Sources Other than Influenza, Pneumococci of All Types, Hemolytic Streptococci |
| Serum 1. Rabbit immunized with coccus from sputum at onset of attack of uncomplicated influenza; coccus not soluble in bile, not agglutinated by pneumococcus serum | | |
| Opsonin | 87% | 0% |
| Agglutinin | 93% | 0% |
| Serum 2. Rabbit immunized with serum from influenza-pneumonia; bile insoluble, not agglutinated by pneumococcus serum | | |
| Opsonin | 90% | 0% |
| Agglutinin | 90% | 0% |
| Serum 3. Rabbits immunized with coccus from influenza-pneumonia; bile insoluble, not agglutinated by pneumococcus serum | | |
| Opsonin | 76% | 0% |
| Agglutinin | 78% | 0% |
| Serum 4. Rabbit immunized with coccus from sputum in influenza-pneumonia; bile insoluble, not agglutinated by pneumococcus serum | | |
| Opsonin | 82% | 0% |
| Agglutinin | 94% | 0% |
| Serum 5. Rabbit immunized with coccus from influenza-pneumonia; bile insoluble, not agglutinated by pneumococcus serum | | |
| Opsonin | 90% | 0% |
| Agglutinin | 83% | 0% |
| Serum 6. Rabbit immunized with coccus of brain of case of influenza-pneumonia; bile insoluble, not agglutinated by pneumococcus serum type 2 | | |
| Opsonin | 85% | 0% |
| Agglutinin | 83% | 8% |
| Serum 7. Rabbit immunized with coccus from influenza-pneumonia; slowly soluble in bile, agglutinated by pneumococcus serum type 3 | | |
| Opsonin | 93% | 0% |
| Agglutinin | 87% | 0% |

Of the strains used to immunize the rabbits, 1, 2, 3, 5 and 7 were isolated at Camp Meade; 4 and 6 in Chicago. Strain 2 was isolated after death from the lung and strain 7 from the blood of the same patient.

Serum 6 agglutinated a strain of pneumococcus, type 2, at a dilution of 1 to 10.

Agglutination was marked with immune rabbit serum at 1:2, but generally no agglutination was observed above 1:20, except with the homologous organisms, and then no agglutination was observed above 1:320. One rabbit agglutinated its own organisms at 1:10 only. Normal rabbit serum did not agglutinate the influenza strains except one which, after long cultivation, was agglutinated at 1:80.

The serum of rabbits 1 and 2 (table 1) were tested with the 34 influenza cocci and 13 strains of cocci from other sources (pneumococci of types 1, 2, 3 and 4; hemolytic streptococci from bronchopneumonia and empyema; and Str. viridans from measles, rubella and bronchitis). The serums of rabbits 3, 4, 5, 6 and 7 (table 1) were tested with 20 influenza cocci and the same strains from other sources. All these immune serums showed a marked increase in opsonins and agglutinins for 86% of the influenza strains. The negative results were obtained with some of the bile soluble strains (pneumococci of types 2 and 4) from Camp Grant. The immune serums showed no increase in opsonins or agglutinins for the cocci from sources other than influenza except that the serum of rabbit 6 agglutinated a pneumococcus of type 2 at a dilution of 1:10. The serum of rabbit 7 immunized with a coccus agglutinated by a pneumococcus type 3 serum, did not agglutinate noninfluenzal pneumococci of type 3.

The results show that the serum of rabbits, immunized with certain green-producing cocci from influenza and its complications, contained opsonins and agglutinins for the influenza strains, whether soluble or insoluble in bile, and whether belonging to the pneumococcus groups or not. Hence, the results indicate that we are dealing with a group of cocci which are closely related immunologically, although certain strains differ in their bile solubility and agglutinability by pneumococcus serums.

Major Fennel of the Army Medical School kindly sent me a large number of strains of pneumococci of type 4 and of Str. viridans isolated from influenza and other sources. The majority of influenza strains were isolated late in the epidemic. With the exception of two strains isolated from spontaneous pneumonias in monkeys, they did not produce colonies similar to those produced by the Mathers coccus and other peculiar green-producing cocci from influenza. No antibodies could be demonstrated for these strains with the 5 immune serums tested, except in these instances: opsonification at a dilution of 1:2 occurred with one influenza strain from Camp Meade and one immune serum; agglutination at 1:2 was demonstrated with two serums and one monkey strain and another Camp Meade influenzal strain. These three strains were type 4 pneumococci. The influenzal history of the two Camp Meade strains is not definite. These results suggest that the strains isolated at the onset and height of the epidemic differed from those isolated later.

### ABSORPTION EXPERIMENTS

Absorption experiments were made to determine whether the agglutination of the allied organisms was due to partial or minor agglutinins. Serum 1 was absorbed with the homologous and four closely related influenzal coccus strains and by pneumococci of types 1 and 2. Killed organisms were suspended in the serum which was incubated for 2 hours, refrigerated for 24 hours, then centrifugated and the supernatant fluid tested. This process was repeated three times when the serum was found no longer to agglutinate the homologous coccus. Absorption with the homologous influenza coccus not only removed the agglutinins for that organism, but also for the 4 closely allied cocci. Absorption with each of the 4 allied influenza cocci, bile insoluble and bile soluble, also absorbed the agglutinins for the homologous coccus as well as for the allied strains, but absorption with pneumococci of types 1 and 2, not of influenzal origin, failed to remove either the agglutinins for the homologous organisms or for the allied strains. These results indicate that these green-producing cocci from influenza and its complications form a group of immunologically closely related organisms.

### COMMENT

The experiments I have described indicate that certain green-producing influenzal cocci are closely related to both pneumococcus and Str. viridans. The bile insolubility of most of these strains and the sugar reactions relate them to streptococci. However, inulin fermentation by these cocci varies. The cocci obtained by Mathers, Rosenow, Howell and Anderson, and Jordan generally did not ferment inulin. Nuzum says his cultures varied a good deal in inulin fermentation. My cultures also varied in this respect. The frequency of lanceolate shape and capsule formation and the type of colony point to their relation to pneumococci. The bile solubility or slow solubility of some of the influenza cocci and the agglutinability of some strains by pneumococcus serums also speak for their close relationship to the pneumococcus. It may be of interest in this connection to refer to certain observations showing that bile-insoluble cocci may be agglutinated by pneumococcus serums and that agglutination with pneumococcus serums is not always specific.

Thus Sutton and Sevier[27] described a Streptococcus mucosus, which was bile insoluble, and morphologically unlike type 3 pneumococcus, but was agglutinated by type 3 serum. During the recent influenza epidemic, Keegan[10] and his co-workers found that some of the fixed pneumococcus types, identified by agglutination, did not show a distinct bile solubility. Mathers[28] and Clough[29] each observed pneumococci which agglutinated equally with types 1 and 2 serums. Blake[30] encountered occasional strains of pneumococci which agglutinated in type 3 serum, and which did not show well developed mucoid characteristics and could not be distinguished culturally from other types of pneumococci. He mentions that there is a small number of strains of pneumococci belonging to type 4 which agglutinate in all 3 types of antipneumococcus serum. In her study of type 4 pneumococci Olmstead[31] observed that groups F and G were agglutinated by both type 4 and type 2 serum. She considers that some members of these groups serve as connecting links between type 2 and type 4, but owing to a closer relationship with the latter they should be classed as of type 4. Recently Clough[32] described 9 strains, three not completely dissolved by bile, which were agglutinated with antipneumococcus serum of types 1, 2 and 3. One strain showed a mutation while under observation. On isolation it had the cultural reactions of a typical pneumococcus and the phagocytic and agglutinative reaction of an atypical type 2 strain. After cultivation it was agglutinated by antipneumococcus serum types 1, 2 and 3 and became bile insoluble, did not ferment inulin and caused precipitation in glucose ascitic fluid agar. She suggests that these pneumococci reacting with all 3 types of antipneumococcus serums may represent primitive, relatively undifferentiated forms from which fixed types may arise. It consequently may be possible that the influenza cocci which I am considering and which have both pneumococcus and streptococcus characteristics form a group between these two forms of cocci.

It does not seem possible to determine definitely the etiologic relation of the peculiar green-producing cocci to influenza and its complications. At Camp Meade the Mathers coccus was the prevailing organism during the onset and height of the epidemic, being present in 87% of the sputums examined. It was the organism which appeared with the onset of influenza—Pfeiffer's bacillus having been present in the respiratory diseases for several weeks previous. The Pfeiffer bacillus was found in 58% of the influenzal sputum cultures. In Chicago, Jordan found this coccus present in a few more cases (66%) than the Pfeiffer bacillus (64%). When present, it was always found in the early days of the attack, and was more closely associated with

[27] Johns Hopkins Hosp. Bull., 1917, 28, p. 315.
[28] Jour. Infect. Dis., 1915, 17, p. 514.
[29] Bull. Johns Hopkins Hosp., 1917, 28, p. 306.
[30] Jour. Exper. Med., 1917, 26, p. 67.
[31] Jour. Immunol., 1917, 2, p. 425.
[32] Jour. Exper. Med., 1919, 30, p. 123.

pneumonia cases than the Pfeiffer bacillus. However, Jordan found the coccus present in colds and tonsillitis in about the same proportion of cases as in influenza, while Pfeiffer's bacillus was found in only 14%. Rosenow also observed this peculiar streptococcus in and about Rochester, Minn., at the very beginning of the epidemic of influenza. K. F. Meyer found the same coccus (personal communication) in 85% of the cases of influenza during the epidemic in California.

My experiments and those of Rosenow and of Howell and Anderson showed that the serum of convalescent influenza and influenzal pneumonia patients contained definite, specific opsonins, agglutinins and complement fixation bodies for these streptococci, which indicates that this coccus played at least some part in the reactions in the epidemic and also that it was of wider distribution than commonly recognized. Unfortunately, human or monkey inoculations and preventive vaccination with this coccus alone were not made during the epidemic.

## SUMMARY

Various investigators isolated peculiar green-producing cocci with the characteristics of both pneumococcus and streptococcus, from influenza and its complications, during the onset and at the height of the recent epidemic. This coccus is oftener lanceolate than round, generally it possesses a capsule, and produces large, moist green colonies on blood-agar plates. It is, as a rule, insoluble in bile, and rarely ferments inulin.

I have isolated this coccus from the edematous brain in influenzal bronchopneumonia, and generally in pure culture. In no instance was the Pfeiffer bacillus cultivated from the brain.

The serum of rabbits immunized with strains of this coccus from influenza and its complications contained opsonins and agglutinins for other similar, bile-insoluble influenzal cocci, and also for certain influenzal cocci, which were bile soluble and agglutinable by antipneumococcus serums. These results indicate that the green producing influenzal cocci form a group, the members of which are closely related immunologically.

The results of absorption experiments with reference to agglutinins also suggest that we are dealing with a group of closely allied organisms. Immune rabbit serum treated with the homologous influenza

coccus lost its agglutinins for the homologous coccus and for allied influenza cocci. Absorption with allied influenza organisms also removed the immune bodies for the homologous coccus as well as for the closely related cocci, but absorption with pneumococci of types 1 and 2 not of influenzal origin, did not remove the agglutinin for the influenza cocci.